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# Tumor-suppressive effect of the retinoic acid receptor β in human epidermoid lung cancer cells

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**ABSTRACT** **Retinoic acid receptor β (RARβ), which codes for a nuclear receptor for retinoic acid, is localized in a chromosomal region frequently deleted in lung cancer cells. The gene is expressed in normal lung tissue and in the majority of the cell lines derived from lung tumors but not in most of the lines derived from lung tumors with epidermoid characteristics. To study the possible role of RARβ in growth control of epidermoid lung tumor-derived cells, transfectants expressing RARβ were generated from nonexpressing epidermoid tumor-derived cell lines. Four clones were derived from line CALU-1, three of which showed a 20–60% increase in doubling time in the presence of retinoic acid. Parental and control-transfected cells were unaffected or slightly stimulated. All four clones expressing RARβ were less tumorigenic in nude mice than were the untransfected or control-transfected cells, with about a 50% incidence of take vs. 95%. When tumors did develop from RARβ-positive cells, they showed a reduced rate of growth, an increased latency, and, in six of seven tumors tested, a much reduced level of RARβ expression. Transfectants derived from a second tumor line, H157, also showed a markedly reduced incidence of take in nude mice. Together with the known effects of retinoic acid on differentiation and carcinogenesis, our results support the hypothesis that RARβ functions as a tumor suppressor gene in epidermoid lung tumorigenesis.**

Lung cancer comprises a group of highly malignant diseases of several distinct histologies. Three types, adenocarcinoma, epidermoid (or squamous), and large cell carcinoma, are considered as a group to be different clinically and biologically from the fourth major class, small cell cancer (1). All types are characterized by a nonrandom heterozygous loss of a region of the short arm of chromosome 3 (3p-) (1). The frequency of this loss has generally been found to be higher in epidermoid tumors than in other non-small cell tumors (2), especially in the distal region (3), suggesting the existence of an epidermoid-specific tumor suppressor gene on 3p (2). This loss has been reported in premalignant squamous dysplasia, suggesting that this change is an early event in epidermoid tumorigenesis (4).

One of the genes in the distal region of 3p is RARβ, which encodes a nuclear retinoic acid receptor (RAR) (5, 6). This gene is of great interest because RA profoundly affects many differentiation and developmental processes (7), and it has been particularly implicated in differentiation and homeostasis of the bronchial epithelium. RA deficiency, which could be functionally equivalent to loss of a copy of a RAR gene, has been associated with bronchial squamous metaplasia in animals (8, 9) and, in humans, with a higher incidence of epidermoid lung cancer (10). Several other genes encoding RARs are known, including RARα, RARγ (11), and another family called RXRs (12), all of which are members of the steroid–thyroid superfamily of receptors. However, none of these genes is as closely associated with nonrandom chromosomal rearrangements in lung cancer as is RARβ.

The circumstantial evidence implicating RARβ in epidermoid tumorigenesis led us (2) and others (13, 14) to analyze RARβ in various cell lines. The gene was not expressed in most cell lines derived from lung tumors with epidermoid characteristics, even though one or more copies of the gene remained intact in the cell. Normal lung tissue and most of the cell lines derived from other types of lung tumors expressed RARβ. The α and γ forms of RARs were expressed in all lung cancer types (2, 14). These results suggested that RARβ is involved in epidermoid lung tumorigenesis and prompted us to test the hypothesis directly. We have transfected an expression vector bearing the RARβ cDNA under the control of a simian virus 40 (SV40) promoter into cell lines derived from epidermoid lung tumors that do not express RARβ. Transfectants expressing RARβ generally grew at reduced rates in the presence of RA and were markedly less tumorigenic than the parental cells when injected into nude mice. Moreover, tumors that did arise from one of the sets of transfectants generally showed a clear reduction in RARβ expression compared to the corresponding cells in culture.

## MATERIALS AND METHODS

**Cell Culture and Transfections.** The epidermoid lung tumor-derived lines CALU-1 and H157 were obtained from the American Type Culture Collection and A. Gazdar (National Cancer Institute, National Institutes of Health), respectively, and were maintained in α-medium supplemented with 10% fetal calf serum (GIBCO) and 1% antibiotics. For transfection, cells were trypsinised, and $10^7$–$10^8$ cells were washed in phosphate-buffered saline (PBS), centrifuged, and washed twice with 5 ml of Opti-MEM medium (GIBCO/BRL). After centrifugation, the pellet was resuspended in the transfection mix consisting of 1.4 ml of Opti-MEM medium, 300 μg of Lipofectin (GIBCO/BRL), 40 μg of pAG60 DNA (15) linearized by *Cla* I, and, where appropriate, 40 μg of pBH-4 linearized by *Pst* I. The latter plasmid was constructed by filling in the ends of the 1.7-kb *Bam*HI–*Hin*dIII fragment of pBLhRARβ (provided by Magnus Pfahl; ref. 5) and cloning in the *Sma* I site of pSVL (Pharmacia), with the SV40 late promoter upstream of the 5′ end of the cDNA sequence. The transfection mix was incubated at 37°C for up to 20 min, diluted into 80 volumes of α-medium supplemented with 10% fetal calf serum, and plated in two 150-mm dishes, to which G418 (GIBCO/BRL; effective concentration, 400 μg/ml) was added after 72 hr. Resistant clones, arising at a frequency

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Abbreviations: RA, retinoic acid; RAR, RA receptor; SV40, simian virus 40.

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of ≈$10^{-6}$ were picked 3–4 weeks later. Cells were shown to be mycoplasma-free using the Hoechst stain kit (ICN–Flow).

**RNA Analysis.** Total RNA was extracted by the lithium chloride/urea method. Briefly, cells (≈4 × $10^7$) were trypsinized, washed with PBS, pelleted, and kept at −80°C. RNA isolation was performed by resuspending each pellet in 3–5 ml of a 3 M LiCl/6 M urea solution, sonicating, and incubating at 4°C overnight. The mixture was then centrifuged, and the pellets were washed with 2 ml of the LiCl/urea solution and then resuspended in 1 ml of 10 mM Tris·HCl, pH 7.6/1 mM EDTA/0.5% SDS. After a phenol/chloroform extraction, RNA was ethanol precipitated. RNA extraction from tumor samples conserved at −80°C was performed the same way after the samples were homogenized in 5 ml of LiCl/urea solution using a Polytron homogenizer (Kinematica, Lausanne, Switzerland). RNase protection assays were performed using 20 μg of RNA, following a standard protocol (2). To detect RARβ sequences, we used a probe generated from *Ava* I-linearized pBH-2 (2), which carries an insert corresponding to the 3′ end of the coding region of RARβ. With this probe, transcripts from the endogenous and transfected RARβ sequences generated protected fragments of different sizes. RNA quantitation was performed by including in the reaction mix a probe derived from *Hin*dIII-linearized pBH-11, which carries a human β-actin *Bam*HI–*Sac* I fragment of 299 bp, cloned in pGEM-1, yielding a protected fragment of 213 bp.

**Protein Analysis.** Fractionation, Western blotting, and immunodetection were performed as described (16). RP(β)F antibody (C.R.-E., unpublished data) was used to detect the RARβ protein. The specificity of the reaction was assessed by using the same antibody depleted by incubation with two peptides covering the F region of RARβ, peptides PB66, and SP176 (17).

**Tumorigenicity Assays.** Cells to be injected were harvested by trypsinization, washed in PBS, counted, and collected by centrifugation, and 5.0 × $10^6$ cells were suspended in 0.3 ml of PBS. They were injected subcutaneously in 4- to 6-week-old *nu/nu* CD-1 mice (Charles River Breeding Laboratories). Tumor volumes were measured weekly using the formula $V = 0.4 \times A \times B^2$, where A and B are the larger and the smaller axis, respectively (18). The latency period was defined as the period preceding the appearance of a tumor >0.05 $cm^3$.

## RESULTS

The CALU-1 line, an epidermoid lung tumor-derived line that was previously shown to be negative for expression of RARβ (2) and tumorigenic in nude mice (19), was initially chosen for transfection experiments. The cells were cotransfected with pAG60 (15), carrying the *neo* gene, which confers resistance

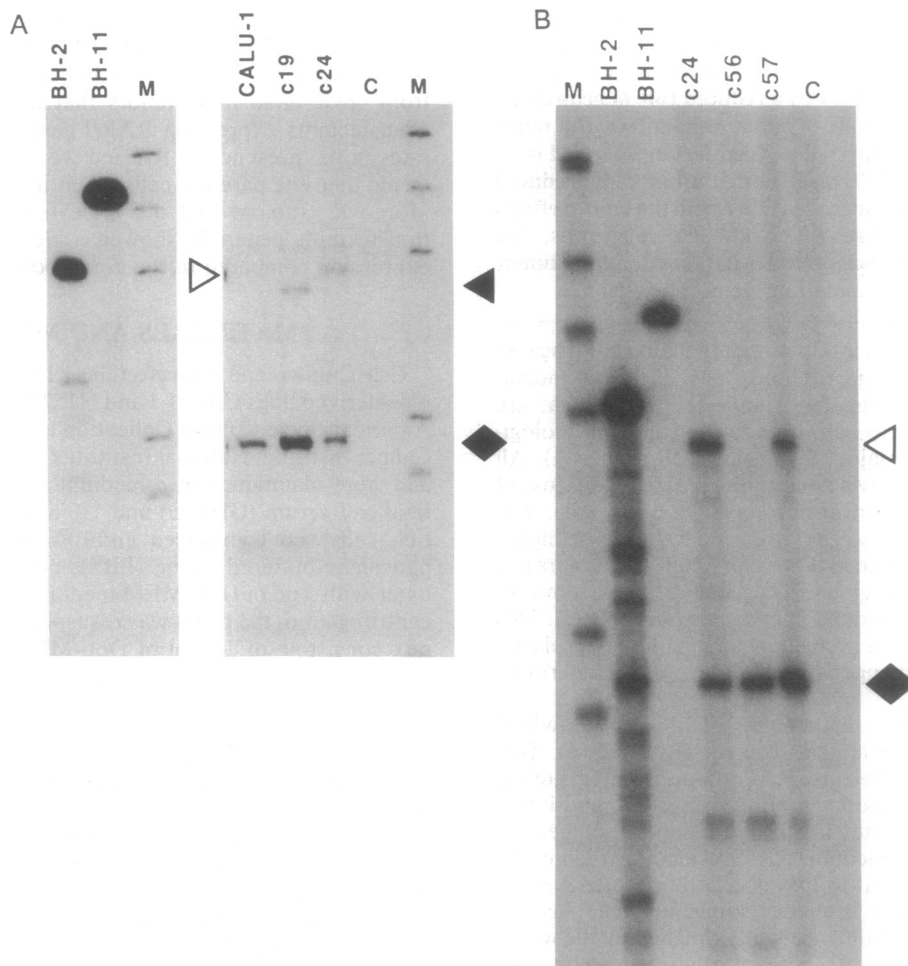


FIG. 1. RNase protection assays to determine expression of endogenous and exogenous RARβ in CALU-1 and several transfected derivatives, c19 and c24 (*A*) and c24, c56, and c57 (*B*). Hybridizations were performed with riboprobes derived from pBH2 (RARβ) and pBH11 (actin control; see text), and RNase protected fragments are indicated by arrowheads and diamonds, respectively. Filled arrowheads indicate the expected band from endogenous RARβ, and open arrowheads indicate the band from exogenous RARβ. Cell line c19 expressed endogenous RARβ, c24 and c57 expressed the exogenous sequence, and CALU-1 and c56 expressed no RARβ. Undigested probes are identified by plasmid of origin; M, marker lanes (BRL ladder); lanes C, tRNA control.

to G418, and pBH-4, bearing the coding sequence of the human RARβ cDNA under the control of the SV40 late promoter (*Materials and Methods*). Among 10 clones tested, 6 were shown to express RARβ. By using a probe permitting the distinction between the endogenous and the exogenous messages in an RNase protection assay (*Materials and Methods*), we found that two of these, c19 and c59, expressed solely or primarily the endogenous RARβ, whereas the other four, c24, c57, c64, and c66, expressed the exogenous sequences (results for three lines shown in Fig. 1).

The reactivation of the endogenous gene in c19 and c59 may have resulted from the interaction between the RA-responsive element in the RARβ promoter (20) and RARβ proteins expressed from exogenous sequences early in the transfection process. Subsequent events, such as DNA rearrangement during chromosomal integration of the plasmid sequences, may explain the lack of expression of the exogenous RARβ at the moment that analysis was performed. We surmise that by this time the level of endogenous RARβ was sufficient to maintain its own transcription. Southern blot data from c19 are consistent with this hypothesis, since the integrated transgene copy in c19 had a rearranged promoter region. We do not understand the details of this interesting phenomenon, but in any event the transfection experiments yielded the desired cell lines that expressed RARβ. A further clone, c30, was isolated after transfection with pAG60 alone. RNase protection assays showed that this line expressed no RARβ (not shown).

The levels of RARβ protein were determined in cell lines CALU-1 and two of the transfectants, c19 and c24, by Western blotting and immunodetection (Fig. 2). The protein, visible as a doublet representing different phosphorylated forms (21), was present in the nuclear fraction of c19 and c24, at about the same level in each, but absent in CALU-1 cells. This was as expected from the results of the RNase protection assays.

The growth rates of several cell lines in culture were determined (Table 1). For three RARβ-positive lines, the rate did not vary markedly from those of CALU-1 or a negative control clone (c30) that was transfected with pAG60 alone. Addition of 0.1 μM RA to the medium, however, retarded the growth of three RARβ-positive lines, increasing doubling time by up to 60%. The only line with noticeably slower growth in the absence of exogenous RA (c64) was also the most affected by RA (Table 1). One of the cell lines tested, c19, expressed endogenous RARβ only (Fig. 1*A*) but was still inhibited to some extent by RA. This suggests that the endogenous gene carries information specifying functional RARβ, at least as measured by this parameter.

Table 1. Growth rates of CALU-1 and transfected derivatives in the presence or absence of RA

| Cell line | RARβ expression | Doubling time, days: Without RA | Doubling time, days: With RA | Ratio* |
|---|---|---|---|---|
| CALU-1 | − | 1.25 | 1.20 | 0.96 ± 0.03 |
| c30 | − | 1.40 | 1.25 | 0.9 ± 0.1 |
| c19 | + | 1.35 | 1.70 | 1.26 ± 0.08 |
| c24 | + | 1.28 | 1.81 | 1.41 ± 0.07 |
| c57 | + | 1.53 | 1.55 | 1.01 ± 0.01 |
| c64 | + | 1.75 | 2.85 | 1.63 ± 0.2 |

Cells were seeded in medium with or without 0.1 μM RA at 5 × $10^4$ per 60-mm Petri dish and duplicate samples were counted either daily or every 2 days. Results of two (c57 and c64) or three (other cell lines) experiments were plotted and doubling times were calculated. *With RA/without RA ± range.

Tumorigenicity was determined by injecting cells into nude mice and a striking effect of RARβ could be seen (Table 2). Nearly all mice injected with CALU-1 or c30 developed nodules within 1–2 weeks. The tumors had a similar growth pattern, typically reaching 0.05 $cm^3$ (usually ≈0.5 cm in diameter) within 6–8 weeks. The growth patterns of tumors derived from the RARβ-positive clones, c19, c24, c57, and c64, were very different, with the latency period usually more than double the RARβ-negative cells, not reaching the threshold 0.05 $cm^3$ until usually 12–20 weeks after injection for those cases in which tumors developed. About one half of these mice, including all four injected with c64, failed to develop any measurable tumor at all. Growth rates of the tumors obtained from RARβ-negative and -positive lines were also different, with average doubling times of 1.5 weeks and about 5 weeks, respectively. This again indicated that RARβ has a tumor-suppressive effect in these cells.

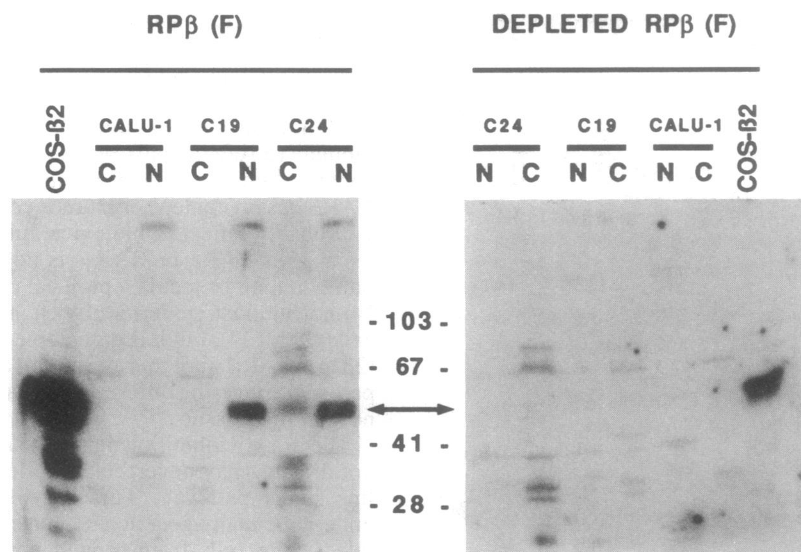


FIG. 2. RARβ protein detected in transfectants. (*Left*) Results of probing cytoplasmic (C) and nuclear (N) fractions of CALU-1 cells and two derived transfectants with RPβ (F) antibody. The expected 51-kDa RARβ protein (double-head arrow) is seen in nuclear extracts of c19 and c24. The two bands represent different phophorylated forms (21). Contamination by nuclear material explains the faint band seen in lane C of c24. (*Right*) Specificity of the reaction is confirmed by absence of the 51-kDa RARβ protein upon probing with the same antibody depleted by incubation with two peptides covering the F region of RARβ (see text). Extracts from COS-1 cells expressing very high levels of the murine RARβ2 were used as positive controls (COS-β2).

Table 2. Tumorigenicity of the CALU-1 cell line and its derivatives

| Cell line | RARβ expression | No. of tumors/ no. of injections | Mean latency, weeks | Mean doubling time, weeks (range) |
|---|---|---|---|---|
| CALU-1 | − | 14/15 | 8.6 | 1.5 (1–2.3) |
| c30 | − | 6/6 | 6.5 | ≈1.2 (1.03–1.5) |
| c19 | + | 7/12 | 19 | 5.6 (3.5–7.0) |
| c24 | + | 6/9 | 16 | 4.1 (2.0–7.0) |
| c57 | + | 2/4 | 13 | 2.7 (2.0–3.5) |
| c64 | + | 0/4 | >16 | — |

Cells ($5 \times 10^6$) were injected subcutaneously into the lumbrosacral region or, in some instances, into the thigh or the cervical region. Tumors were measured weekly. Injections scored as negative were monitored for ≥15 weeks, except for one of c19, two of c24, and one of c57, which were followed for between 11 and 13 weeks.

If this is so, one would expect that the phenotype of the tumors generated from transfected cells may reflect selective pressure against RARβ expression. This was tested directly by measuring the level of RARβ message in tumors. Four c24-derived tumors were therefore excised and relative RARβ expression was determined using β-actin as an internal control. The results for three tumors (series T c24) are shown in Fig. 3, and a striking reduction in RARβ message levels is seen compared with the corresponding clone c24 (lane 12). Similar results were obtained for the fourth tumor (not shown). Densitometry showed that reductions were >10-fold in all four cases. We also analyzed three c19-derived tumors, and two of these had about a 5-fold lower level of RARβ message (one shown in lane 5; compare with lane 11). The third had a marginally higher level than the corresponding cultured cells (lane 4). As expected, CALU-1-derived tumors expressed no detectable RARβ message (lanes 6–10). Thus, as has been concluded from similar data with other tumor-suppressor genes (22, 23), there has in all likelihood been a selection against expression of the gene responsible for control of *in vivo* growth in six of the seven tumors examined. The one exceptional case probably represents reacquisition of tumorigenic potential by means of any of a number of mechanisms not involving reduced RARβ transcription. These include mutation of the RARβ sequences or events affecting other genes in the RARβ-mediated regulatory pathway.

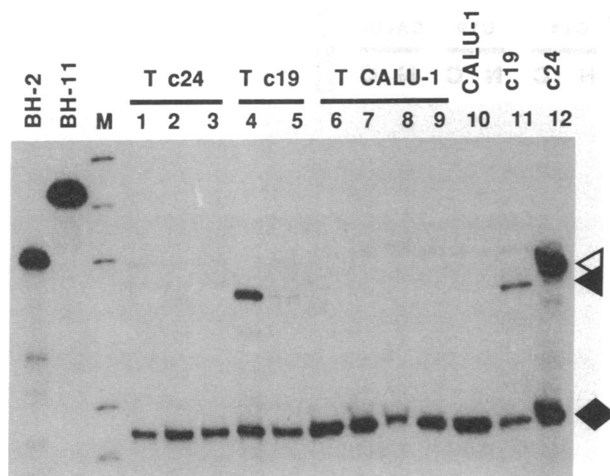


FIG. 3. RNase protection assays to determine relative expression levels of RARβ in various tumors and their corresponding cell lines. Probes and symbols indicating the expected protected fragments are as for Fig. 1. T c24 (lanes 1–3) signifies tumors derived from c24; T c19 (lanes 4 and 5), from c19; etc. Lanes 10–12, cell lines.

Table 3. Tumorigenicity of the H157 cell line and its derivatives

| Cell line | No. of tumors/ no. of injections | Mean latency, weeks |
|---|---|---|
| H157 | 6/6 | 4.7 |
| c223 | 5/6 | 4.7 |
| c226 | 3/10 | 10.8 |
| c228 | 0/6 | — |
| c232 | 1/4 | 9.3 |
| c236 | 4/6 | 7.4 |
| c237 | 2/8 | 4.7 |

Cells ($5 \times 10^6$) were injected as described in Table 1. Mice were observed for >20 weeks.

The cell line H157 was also cotransfected with pBH-4 and pAG60. Protection analyses using the same probe as in Fig. 1 showed that clones expressing RARβ had all activated the endogenous gene, as was seen for c19 and c59. Six clones that expressed RARβ were analyzed for tumorigenic potential (Table 3). Overall, 15 of 40 injections of transfected cells yielded tumors in nude mice, compared with 6 of 6 for the parental line. In addition, mean latency of the tumors that did appear was substantially longer for RARβ-expressing cells, being increased by up to 2-fold or more. In general, only one of the six transfected lines (c223) was at all similar to the parental line with respect to tumorigenicity and interestingly, this particular line only expressed a trace of RARβ (data not shown).

Histological analyses were performed on tumors arising from several of each set of transfectants as well as from parental lines. In all cases, a poorly differentiated epidermoid histology was observed. This result is not unexpected since, at least for CALU-1 derivatives, the tumors tended to have low RARβ expression and in this way resembled the parental line.

## DISCUSSION

The results presented here show that RARβ has a role in growth control of epidermoid lung tumor cell lines. First, we have demonstrated that RA has a growth-inhibitory effect on derivatives of CALU-1 cells that express RARβ but not on the parental cells. Such inhibition of cell growth in culture is a characteristic of a number of genes with tumor suppressor activity. For example, p53 transfected into tumor cells can arrest growth by blocking progression from $G_0/G_1$ to S phase (24), and the RB-1 gene confers density-dependent growth inhibition on bladder tumor cells (25). These activities are thought to reflect roles played by these genes in cell cycle control. Similarly, the RA-dependent inhibitory effect that RARβ has on epidermoid tumor cell growth in culture (Table 1) probably reflects a biological function of this gene. RARβ (as against RARα or RARγ) is not expressed in undifferentiated human bronchial epithelial cells growing in culture (2, 14) nor in most epidermoid (squamous) lung cancer-derived cells (2, 13, 14), but it is detectable in total normal lung tissue (2, 14). RARβ may therefore play a critical role in the signal pathway triggering differentiation and limiting cell division in normal lung tissue.

The growth inhibition demonstrated here (Table 1), though not drastic, may nevertheless reflect a partial functioning of this signal pathway. Tumor cells such as CALU-1 have undergone many genetic and epigenetic changes that tend to release the cells from growth control mechanisms, so the molecular context in which the RARβ-triggered signal pathway operates in our transfected CALU-1 cells would be substantially altered from that in normal bronchial epithelium. In this light it is not unreasonable that the simple introduction of functional RARβ may not completely restore RA responsiveness. Furthermore, other isoforms of RARβ

that are generated by alternative splicing in murine cells (26, 27), at least some of which exist in human cells (B.H., M. Pelletier, and W.E.C.B., unpublished data), may play a contributory role in this regard, and it will be important to assess the combined effect of all RARβ isoforms in this system.

We have also demonstrated an inhibitory effect of RARβ on the tumor-forming ability of CALU-1 and H157 cells. All parameters of tumor growth—incidence of take, latency, and growth rate—were affected in virtually all RARβ-expressing transfectants analyzed. Furthermore, in most cases in which tumors were analyzed, RARβ expression was greatly or at least substantially reduced. All of these observations argue in favor of a role for RARβ in tumor suppression. This effect may be in part due to the RA-dependent inhibitory effect on growth observed in culture, since the *in vivo* environment presumably contains RA. However, this cannot satisfactorily explain the full *in vivo* effect, since the concentrations of the ligand at which we detected the inhibitory effect are >100-fold higher than physiological levels. When 1 nM RA was tested, inhibition was only 14% for c24 and negligible for c19 (data not shown). In addition, cell line c57 was unaffected by RA in culture, even at 0.1 μM, but still had much reduced tumorigenicity. Thus RARβ decreased the tumor potential of the CALU-1 cell line by mechanisms other than simple growth inhibition.

What could these mechanisms involve? We cannot yet answer this question definitively, but recent work documenting the interactions between RARs and other transcription factors may provide some hints. For example, RARs and AP1 (a complex formed by *jun–jun* and *jun–fos* dimers, ref. 28) can act antagonistically to each other (29, 30). This antagonism has been directly implicated in the regulation of collagenase (30) and stromelysin (29), two genes that play key roles in tumor potential and invasiveness by allowing remodeling of the extracellular matrix. In our system, RARβ may be acting by negatively regulating genes such as collagenase and stromelysin, whose action depends upon, or is directed toward, elements of the *in vivo* environment. Such a negative effect would not be manifested in cells growing in culture since these elements are absent. Therefore, RARβ may act as a tumor-suppressor gene by more than one mechanism.

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1. Carney, D. N. & De Leij, L. (1988) *Semin. Oncol.* **15**, 199–214.
2. Houle, B., Leduc, F. & Bradley, W. E. C. (1991) *Genes Chromosomes Cancer* **3**, 358–366.
3. Yokoyama, S., Yamakawa, K., Tsuchiya, E., Murata, M., Sakiyama, S. & Nakamura, Y. (1992) *Cancer Res.* **52**, 873–877.
4. Sundaresan, V., Ganly, P., Hasleton, P., Rudd, R., Sinha, G., Bleehan, N. M. & Rabitts, P. (1992) *Oncogene* **7**, 1989–1997.
5. Benbrook, D., Lernhardt, E. & Pfahl, M. (1988) *Nature (London)* **333**, 669–672.
6. Brand, N., Petkovich, M., Krust, A., Chambon, P., de Thé, H., Marchio, A., Tiollais, P. & Dejean, A. (1988) *Nature (London)* **332**, 850–853.
7. Roberts, A. B. & Sporn, M. B. (1984) in *The Retinoids*, eds. Sporn, M. B., Roberts, A. B. & Goodman, D. S. (Academic, Orlando, FL), Vol. 2, pp. 209–286.
8. Wolbach, S. B. & Howe, P. R. (1925) *J. Exp. Med.* **42**, 753–777.
9. Harris, C. C., Silverman, T., Smith, J. M., Jackson, F. & Boren, H. G., J. (1973) *J. Natl. Cancer Inst.* **51**, 1059–1062.
10. Graham, S. J. (1984) *J. Natl. Cancer Inst.* **73**, 1423–1428.
11. Green, S. & Chambon, P. (1988) *Nature (London)* **324**, 615–617.
12. Mangelsdorf, D. J., Ong, E. S., Dyck, J. A. & Evans, R. M. (1990) *Nature (London)* **345**, 224–229.
13. Nervi, C., Vollberg, T. M., George, M. D., Zelent, A., Chambon, P. & Jetten, A. (1991) *Exp. Cell Res.* **195**, 163–170.
14. Gebert, J. F., Moghal, N., Frangioni, J. V., Sugerbaker, D. J. & Neel, B. G. (1991) *Oncogene* **6**, 1859–1868.
15. Colbère-Garapin, F., Horodniceanu, F., Kourilsky, P. & Garapin, A.-C. (1981) *J. Mol. Biol.* **150**, 1–14.
16. Rochette-Egly, C. (1991) *J. Cell Biol.* **15**, 535–545.
17. Gaub, M. P., Lutz, Y., Ruberte, E., Petkovich, M., Brand, N. & Chambon, P. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3089–3093.
18. Attia, M. A. M. & Weiss, D. W. (1966) *Cancer Res.* **26**, 1787–1800.
19. Fogh, J., Fogh, J. M. & Orfeo, T. (1977) *J. Natl. Cancer Inst.* **59**, 221–226.
20. de Thé, H., Vivanco-Ruiz, M. D. M., Tiollais, P., Stunnerberg, H. & Dejean, A. (1990) *Nature (London)* **343**, 177–180.
21. Rochette-Egly, C., Gaub, M.-P., Lutz, Y., Ali, S., Scheuer, I. & Chambon, P. (1992) *Mol. Endocrinol.*, in press.
22. Frisch, S. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9077–9081.
23. Bookstein, R., Shew, J.-Y., Chen, P.-L., Scully, P. & Lee, W.-H. (1990) *Science* **247**, 712–715.
24. Mercer, W. E., Shields, M. T., Amin, M., Sauve, G. J., Appella, E., Romano, J. W. & Ullrich, S. J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6166–6170.
25. Takahashi, R., Hashimoto, T., Xu, H.-J., Hu, S.-X., Matsui, T., Miki, T., Bigo-Marshall, H., Aaronson, S. A. & Benedict, W. P. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5257–5261.
26. Zelent, A., Mendelsohn, C., Kastner, P., Krust, A., Garnier, J.-M., Ruffenach, F., Leroy, P. & Chambon, P. (1991) *EMBO J.* **10**, 71–81.
27. Nagpal, S., Zelent, A. & Chambon, P. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2718–2722.
28. Karin, M. (1990) *Mol. Aspects Cell. Regul.* **6**, 143–161.
29. Nickholson, R. C., Mader, S., Nagpal, S., Leid, M., Rochette-Egly, C. & Chambon, P. (1990) *EMBO J.* **9**, 4443–4454.
30. Yang-Yen, H.-F., Zhang, X.-K., Graupner, G., Tzukerman, M., Sakamoto, B., Karin, M. & Pfahl, M. (1991) *New Biol.* **3**, 1206–1219.